Optimization of Chromatography Makes A Breakthrough of Radical Identification in Lipid Peroxidation: LC-ESR, LC-MS, and MS-MS Characterizes All Expected POBN Lipid-Derived Adducts

Steven Y. Qian, Qiong Guo, Gui-Hua Yue, Kenneth B. Tomer, and Ronald P. Mason

Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, NC, 27709

An on-line high performance liquid chromatography (LC)-electron spin resonance (ESR) system combined with tandem mass spectrometry (MS-MS) created a unique technique to identify a variety of lipid-derived radicals (•Ld) formed from in vitro lipid peroxidation [1]. To improve the sensitivity, resolution, and reliability of this method for in vitro and in vivo studies [2-3], we investigated the effects of pH, modifier, and column on chromatographic separation of lipid-derived radicals from the reaction of linoleic acid and soybean lipoxygenase in presence of the spin trap α-[4-pyridyl 1-oxide]-N-*tert*-butyl nitrone (POBN). Chromatographic resolution was greatly improved by: (1) removing NH4C2H3O2 from mobile phase; (2) selecting THF as a new modifier; and (3) using an acidic mobile phase (0.1% HOAc), thus increasing retention reproducibility of lipid-derived radical adducts. In addition, these changes allowed us to eliminate an ESR tuning problem and to synchronize the detection of radical adducts with UV and ESR in on-line LC-ESR, neither of which had been previously possible. The improved resolution and sensitivity of radical detection significantly increased the method's reliability and finally made possible a breakthrough in radical identification via combination of LC-ESR and LC-MS. The peroxidations of five polyunsaturated fatty acids (PUFAs: linoleic aicd, linolenic acid, arachidonic acid, EPA, and DHA) were tested by on-line LC-ESR and on-line LC-MS using the optimized chromatographic procedure. The POBN adducts of all expected lipid-derived radicals generated from PUFAs were selectively detected by LC-ESR, synchronously identified by LC-MS, and structurally characterized by Tandem MS. These lipid-derived carbon-centered radicals, including lipid alkyl radicals (L•), epoxyallyic acid radicals (OL•), dihydroxyallyic acid radicals (OL• (H2O)), and b-scission radicals (R•/•RCOOH) from the different lipid alkoxyl radicals, gave distinct retention times: OL• (H2O) ~ 4-6 min, R• and •R-COOH ~7-23 min, L• and OL• ~ 25-36 min. The application of this method in the identification of lipid-derived radicals formed in vivo and cellular membranes were also illustrated.

[1] Hideo Iwahashi, Leesa J. Deterding, Carol E. Parker, Ronald R. Mason, and Kenneth B. Tomer. Free Rad. Res., 25, 255-27, 1996
[2] Steven Y. Qian, and Ronald R. Mason. Free Rad. Biol. Med. 31, S13, 2001
[3] Steven Y. Qian, Yeong R. Chen, Leesa J. Deterding, Yang C. Fann, Colin F. Chignell, Kenneth B. Tomer, and Ronald R. Mason, Biochem. J. In press. (2001)